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Platelet Glycoprotein IIb/IIIa Pl^{A2}/Pl^{A2} Homozygosity Associated With Risk of Ischemic Cardiovascular Disease and Myocardial Infarction in Young Men

The Copenhagen City Heart Study

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OBJECTIVES We tested the hypothesis that platelet glycoprotein (GP) IIb/IIIa Pl^{A2}/Pl^{A2} homozygotes or Pl^{A1}/Pl^{A2} heterozygotes versus Pl^{A1}/Pl^{A1} noncarriers have increased risk of ischemic cardiovascular disease and myocardial infarction (MI), stratified for age and gender.

BACKGROUND The GP IIb/IIIa Pl^{A1}/Pl^{A2} polymorphism influences aggregation of platelets; however, an association between ischemic cardiovascular disease and heterozygosity remains controversial, and association with homozygosity is largely unexplored.

METHODS We genotyped the participants of the Copenhagen City Heart Study, a prospective cardiovascular investigation of the Danish general population (n = 9,149, 22-year follow-up) and assessed the risk of ischemic cardiovascular disease in heterozygotes or homozygotes versus noncarriers.

RESULTS Of the participants, 70.0%, 27.3%, and 2.7% were noncarriers, heterozygotes, or homozygotes, respectively. Incidence of ischemic cardiovascular disease was 167 and 103 per 10,000 person-years in homozygous and noncarrier men (log-rank: p = 0.006), whereas this difference was not observed in women (p = 0.33) (genotype·gender interaction: p = 0.03). In homozygous versus noncarrier men <40 years of age, 40 to 50 years, and >50 years at entry, age-adjusted relative risks (RRs) of ischemic cardiovascular disease were 3.6 (1.4 to 9.0), 2.4 (1.3 to 4.6), and 1.0 (0.6 to 1.8), respectively (age·genotype interaction in men: p = 0.04); equivalent multifactorially adjusted RRs were 3.0 (1.1 to 8.0), 2.0 (1.0 to 3.9), and 1.0 (0.6 to 1.8), respectively. The corresponding age-adjusted RR values of MI in men were 5.2 (1.5 to 18), 3.5 (1.6 to 7.5), and 0.5 (0.1 to 1.5), respectively (age·genotype interaction in men: p = 0.002); equivalent multifactorially adjusted RRs were 3.8 (1.0 to 15), 3.1 (1.4 to 6.9), and 0.5 (0.2 to 1.5), respectively.

CONCLUSIONS Pl^{A2}/Pl^{A2} homozygosity is associated with a three-fold and four-fold risk of ischemic cardiovascular disease and MI in young men. (J Am Coll Cardiol 2003;42:661–7) © 2003 by the American College of Cardiology Foundation

Binding of adhesive plasmaproteins as von Willebrand factor and fibrinogen to membrane-bound glycoprotein (GP) IIb/IIIa of platelets plays a pivotal role in platelet aggregation (1). The Pl^{A1}/Pl^{A2} polymorphism, also called HPA-1a/HPA-1b or Zw(a)/Zw(b), causes a substitution of the wild-type Leu-33 to proline in the β_3-subunit of GP IIb/IIIa, resulting in an extracellularly positioned conformational change of the β_3-subunit. Therefore, it has been considered biologically plausible to suggest an impact of Leu33Pro on platelet aggregation (2), and consequently on risk of ischemic cardiovascular disease (3).

In a recent meta-analysis (4) of mainly case-control studies with conflicting results encompassing more than 17,000 individuals, aggregated results showed an odds ratio for ischemic cardiovascular disease in Pl^{A1}/Pl^{A2} heterozygotes combined with Pl^{A2}/Pl^{A2} homozygotes versus Pl^{A1}/Pl^{A1} noncarriers of 1.10 (95% confidence interval [CI] 1.03 to 1.18). Only two prospective studies have been published so far (5,6), and all studies published have been too small to specifically investigate homozygosity, let alone having been stratified for age and gender.

We tested the hypothesis that platelet GP IIb/IIIa homozygotes or heterozygotes versus noncarriers have increased risk of ischemic cardiovascular disease and myocardial infarction (MI), overall or stratified for age and gender. For this purpose we used the Copenhagen City Heart Study, a prospective cardiovascular study of the Danish general population with 9,149 participants and a total of 22 years' follow-up.

METHODS

From the Danish general population, by use of the Danish Central Population Register number, we randomly recruited 4,082 men and 5,067 women who participated in the third examination of the Copenhagen City Heart Study, 1991 to 1994 (7–12). Participants were screened for manifestations

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Abbreviations and Acronyms

CI = confidence interval
GP = glycoprotein
ICD = International Classification of Diseases
MI = myocardial infarction
RR = relative risk

of ischemic cardiovascular disease (MI, non-MI ischemic heart disease, or ischemic stroke) by a questionnaire during 1976 to 1978, 1981 to 1983, and 1991 to 1994, and by reviewing all 1976 to 1998 hospital admissions and diagnoses entered in the Danish National Hospital Discharge Register (13), all 1991 to 1998 causes of deaths entered in the Danish National Register of Causes of Death, and medical records from hospitals and general practitioners.

Myocardial infarction was classified according to World Health Organization International Classification of Diseases 8th ed. (ICD-8) code 410, or 10th ed. (ICD-10) codes I21-I22. Non-MI ischemic heart disease was ICD-8 codes 411-413 or ICD-10 codes I20 and I25: diagnosis was based on characteristic symptoms of stable angina pectoris according to the guidelines of the European Society of Cardiology (location, character, and duration of pain and the relation of pain to exercise [14]).

Ischemic stroke was ICD-8 codes 432-434 or ICD-10 code I63, excluding computed tomography proven hematoma, subarachnoidal hemorrhage, or transient ischemic attack. Individual diagnoses were verified by experienced cardiologists and neurologists, respectively (15). Participants with disease before study entry were excluded from the study (n = 93).

We had 99.97% follow-up; three individuals were lost. More than 99% were whites of Danish descent. All participants gave written informed consent. The ethical committees of Copenhagen and Frederiksberg approved the study (No. 100.2039/91).

The PI^{A2}-allele, also called HPA-1b or Zw(b), is caused by a T→C substitution in nucleotide 176 after A in the start-codon in the gene integrin β_3 (GenBank AccNo NM_000212). This polymorphism was examined as earlier described (16): in short, a 268-bp polymerase chain reaction-fragment covering the whole of exon 3 (GenBank AccNo M32672) was amplified from genomic deoxyribonucleic acid by the use of intronic primers: sense: TTCTGATTGCTGGACTTCTCTT; antisense: TCTCTCCCCACGGCAAAGAGT. After thermocycling, the polymerase chain reaction was digested with the restriction endonuclease MspI, run on a 3% agarose gel and visualized using ethidium bromide. The PI^{A1}/PI^{A1} noncarrier genotype produced a 221/38/8-bp-pattern, PI^{A1}/PI^{A2} heterozygous genotype a 221/177/44/38/8-bp-pattern, and PI^{A2}/PI^{A2} homozygous genotype a 177/44/38/8-bp-pattern.

Blood samples were drawn in the nonfasting state. Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, high-density lipoprotein cholesterol, triglycerides, fibrinogen (all Boehringer Mannheim, Mannheim, Germany), and lipoprotein(a) total mass (DAKO A/S, Glostrup, Denmark). The diagnosis of diabetes mellitus was assigned according to participants' own knowledge of their disease status. The status of smoker was assigned to current and former smokers.

The statistical software package SPSS (SPSS for Windows, release 10.0.7, SPSS Inc., Chicago, Illinois) was used. A p value <0.05 on a two-sided test was considered significant. For characteristics of participants, we used the Student *t* test on untransformed or log-transformed variables, Mann-Whitney *U* test, or the Pearson chi-square test. Multifactorial adjustment included all other parameters listed in Table 1 in an analysis of covariance or logistic regression model. Continuous variables were divided in gender-specific tertiles. It was decided a priori to stratify main analyses for gender and age. We plotted cumulative disease incidence against follow-up time using the Kaplan-Meier method, and we tested differences between genotypes using log-rank statistics. Visual inspection of log-minus-log curves was employed to exclude disproportion of hazards over time. Relative risk for disease with 95% CI was calculated using the Cox regression, unadjusted, adjusted for age at entry in decades, or all the factors listed in Table 1 (multifactorial adjustment). We tested for multiplicative interactions among gender, age, and genotype-status in predicting disease, by introducing two-factor interaction terms after inclusion of individual parent terms in the Cox regression model.

RESULTS

We genotyped 9,149 participants from the Danish general population. Of those, 70.0%, 27.3%, and 2.7% were PI^{A1}/PI^{A1} noncarriers, PI^{A1}/PI^{A2} heterozygotes, and PI^{A2}/PI^{A2} homozygotes, respectively. This distribution did not differ from Hardy-Weinberg equilibrium (chi-square test: all, p = 0.81; men, p = 0.97; women, p = 0.77; Table 2). Allele frequencies of the PI^{A1}-allele and PI^{A2}-allele were 83.6/16.4% overall, 83.6/16.4% in men, and 83.7/16.3% in women. Established risk factors for ischemic cardiovascular disease, except for fibrinogen in men, diabetes and hypertension in women, and lipoprotein(a) in both genders, were evenly distributed among the three genotypes (Table 1). Most of these differences were likely due to chance, and none would remain significant after correction for multiple comparison. In contrast, many differences existed in risk factor distribution between men and women (Table 1).

Incidence of ischemic cardiovascular disease in homozygous and noncarrier men was 167 and 103 per 10,000 person-years (log-rank: p = 0.006) (Fig. 1, Table 2). In an age-adjusted Cox regression model, RR of ischemic cardiovascular disease in homozygotes versus noncarriers was 1.5 (95% CI 1.1 to 2.2) and 0.7 (95% CI 0.4 to 1.4) in men and women, respectively (Table 2). In accordance with this gender difference, gender and (homozygous vs. noncarrier) genotype interacted on ischemic cardiovascular disease (p =

Table 1. Characteristics of Participants

	Noncarriers (PI^{A1}/PI^{A1})		Heterozygotes (PI^{A1}/PI^{A2})		Homozygotes (PI^{A2}/PI^{A2})	
Men						
No. (%)	2,854 (68.7)		1,118 (28.6)		110 (2.6)	
Age at entry (yrs)	44.5 ± 0.21		44.7 ± 0.35		45.5 ± 1.08	
Fibrinogen (mg/l)	3.06 ± 0.02		3.07 ± 0.03		3.33 ± 0.11*§	
Body mass index (kg/m^2)	26.1 ± 0.07		26.2 ± 0.12		26.3 ± 0.40	
Cholesterol (mmol/l)	6.0 ± 0.02		5.9 ± 0.04		6.0 ± 0.11	
HDL cholesterol (mmol/l)	1.38 ± 0.01		1.37 ± 0.01		1.40 ± 0.04	
Lipoprotein(a) (mg/l)	297 ± 7		278 ± 10*‡		273 ± 30	
Triglycerides (mmol/l)	2.1 ± 0.04		2.2 ± 0.08		2.1 ± 0.13	
Smoking (%)	82.2		81.3		85.5	
Antihypertensive medication (%)	10.3		10.1		10.0	
Cholesterol-lowering medication (%)	0.9		1.3		2.8	
Diabetes mellitus (%)	4.5		4.3		4.5	
		p Values vs. Men		**p Values vs. Men**		**p Values vs. Men**
Women						
No. (%)	3,550 (70.1)	0.90	1,379 (27.2)	0.87	138 (2.7)	0.93
Age at entry (yrs)	45.8 ± 0.20	0.000	46.0 ± 0.31	0.001	46.2 ± 0.89	0.64
Fibrinogen (mg/l)	3.11 ± 0.02	0.004	3.15 ± 0.02	0.01	3.09 ± 0.08	0.11
Body mass index (kg/m^2)	25.1 ± 0.08	0.000	25.2 ± 0.13	0.000	25.7 ± 0.47	0.05
Cholesterol (mmol/l)	6.3 ± 0.02	0.000	6.3 ± 0.04	0.000	6.2 ± 0.11	0.11
HDL cholesterol (mmol/l)	1.73 ± 0.01	0.000	1.72 ± 0.01	0.000	1.78 ± 0.05	0.000
Lipoprotein(a) (mg/l)	322 ± 7	0.001	334 ± 11‡	0.000	268 ± 28	0.95
Triglycerides (mmol/l)	1.7 ± 0.02	0.000	1.7 ± 0.03	0.000	1.6 ± 0.07	0.30
Smoking (%)	68.3	0.005	70.3	0.000	65.0	0.000
Antihypertensive medication (%)	10.3	0.99	10.1	0.98	17.4†‡	0.10
Cholesterol-lowering medication (%)	0.5	0.13	1.3†	0.96	1.5	0.80
Diabetes mellitus (%)	2.4	0.000	2.1	0.002	5.1*	0.85

Values are means ± SE or frequencies. Heterozygotes and homozygotes were compared with noncarriers of the same gender using the Mann-Whitney *U* test, the Pearson chi-square test, or Student *t* test on untransformed or log-transformed parameters. *$p < 0.05$. †$p < 0.01$. Equivalent multifactorial adjusted comparison including all other covariants listed in the Table using analysis of covariance or logistic regression: ‡$p < 0.05$. §$p < 0.01$. Women and men were compared using the Mann-Whitney *U* test, the Pearson chi-square test, or Student *t* test on untransformed or log-transformed parameters.

HDL = high-density lipoprotein.

0.03). For the end point of MI, the results were similar to those for ischemic cardiovascular disease; however, statistical significance was not reached (Table 2). Heterozygotes did not differ from noncarriers with respect to incidence or risk of ischemic cardiovascular disease or MI in either gender (Table 2). Unadjusted and multifactorial adjusted models gave results similar to age-adjusted models (Table 2).

In homozygous versus noncarrier men aged <40 years, 40 to 50 years, and >50 years at entry, age-adjusted RR values of ischemic cardiovascular disease were 3.6 (1.4 to 9.0), 2.4 (1.3 to 4.6), and 1.0 (0.6 to 1.8), respectively (genotype·age interaction in men: $p = 0.04$; Table 3); equivalent multifactorially adjusted RR values were 3.0 (1.1 to 8.0), 2.0 (1.0 to 3.9), and 1.0 (0.6 to 1.8), respectively. The corresponding age-adjusted RRs of MI in men were 5.2 (1.5 to 18), 3.5 (1.6 to 7.5), and 0.5 (0.1 to 1.5), respectively (age·genotype interaction in men: $p = 0.002$); equivalent multifactorially adjusted RRs were 3.8 (1.0 to 15), 3.1 (1.4 to 6.9), and 0.5 (0.2 to 1.5), respectively. In women, homozygosity versus noncarrier status did not predict ischemic cardiovascular disease or MI in either age group (Table 3). Likewise, heterozygosity versus noncarrier status did not predict either end point in either age group in men or women. Unadjusted and multifactorial adjusted models gave results similar to age-adjusted models (Table 3).

Fibrinogen, a well-recognized predictor of MI (17,18) directly interacting with platelet GP IIb/IIIa in aggregation of platelets, is in our study higher among PI^{A2}/PI^{A2} men than among PI^{A1}/PI^{A2} heterozygous or PI^{A1}/PI^{A1} noncarrier men (Table 1). Accordingly, fibrinogen level could confound the association between disease and homozygosity in men; however, after multifactorial adjustment (including fibrinogen), the RR for homozygous men remained elevated (Tables 2 and 3).

Age at first MI was 61 ± 2.5 years (mean ± SE) and 66 ± 0.5 years in homozygous and noncarrier men (Table 4; Mann Whitney *U* test: $p = 0.03$); equivalent values for ischemic cardiovascular disease were 64 ± 2.0 and 67 ± 0.5 years ($p = 0.07$). This difference was not observed between homozygotes and noncarrier women, or between heterozygotes and noncarriers of either gender (Table 4).

The unadjusted RRs of death due to MI with seven years of follow-up (1991 to 1994 through 1998) for noncarriers, heterozygotes, and homozygotes were 1.0, 0.8 (95% CI 0.5 to 1.3), and 1.8 (95% CI 0.7 to 5.0) for men and 1.0, 1.0 (95% CI 0.5 to 2.2), and 1.1 (95% CI 0.2 to 8.4) for women,

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Table 2. Incidence and Relative Risk of Ischemic Cardiovascular Disease and Myocardial Infarction in PI^{A1}/PI^{A2} Heterozygotes or PI^{A2}/PI^{A2} Homozygotes Versus PI^{A1}/PI^{A1} Noncarriers by Cox Regression Analysis

			Ischemic Cardiovascular Disease					Myocardial Infarction				
	Participants (n)	Hardy-Weinberg Equilibrium	First Events (n)	Incidence (95% CI)/10,000 Person-Years	Relative Risk (95% CI) Adjustment: None	Age	Multifactorial	First Events (n)	Incidence (95% CI)/10,000 Person-Years	Relative Risk (95% CI) Adjustment: None	Age	Multifactorial
Men												
Noncarriers	2,854	p = 0.97	470	103 (95–114)	1.0	1.0	1.0	215	43 (38–49)	1.0	1.0	1.0
Heterozygotes	1,118		190	107 (93–124)	1.1 (0.9–1.2)	1.1 (0.9–1.3)	1.0 (0.9–1.2)	88	45 (36–56)	1.1 (0.8–1.4)	1.1 (0.8–1.4)	1.0 (0.8–1.4)
Homozygotes	110		29	167 (112–239)	1.7 (1.2–2.4)	1.5 (1.1–2.2)	1.5 (1.0–2.1)	13	67 (36–115)	1.6 (0.9–2.7)	1.4 (0.8–2.5)	1.4 (0.8–2.5)
Women												
Noncarriers	3,550	p = 0.77	347	58 (52–65)	1.0	1.0	1.0	117	18 (15–22)	1.0	1.0	1.0
Heterozygotes	1,379		139	58 (49–69)	1.0 (0.8–1.2)	1.0 (0.8–1.2)	0.9 (0.8–1.2)	45	17 (13–23)	1.0 (0.7–1.3)	0.9 (0.7–1.3)	0.8 (0.6–1.2)
Homozygotes	138		10	43 (21–79)	0.7 (0.4–1.4)	0.7 (0.4–1.4)	0.6 (0.3–1.2)	3	12 (2–35)	0.7 (0.2–2.1)	0.7 (0.2–2.0)	0.6 (0.2–2.0)
Gender-genotype interaction test												
Heterozygotes vs. noncarriers					p = 0.62	p = 0.59	p = 0.67			p = 0.65	p = 0.59	p = 0.39
Homozygotes vs. noncarriers					p = 0.02	p = 0.03	p = 0.02			p = 0.16	p = 0.20	p = 0.17

Multifactorial adjustment included all factors listed in Table 1.
CI = confidence interval.

Figure 1. Kaplan-Meier curves showing 22-year cumulative incidence of ischemic cardiovascular disease according to PI^{A} genotype for men and women combined, men alone, and women alone.

respectively. Only 81 (59 noncarrier, 18 heterozygous, and 4 homozygous) men and 33 (23 noncarrier, 9 heterozygous, and 1 homozygous) women died of MI during this short period; therefore, the statistical power is much less in these analyses on mortality compared with the analyses on morbidity.

DISCUSSION

This study reports an increased risk of ischemic cardiovascular disease and MI in young men homozygous for the

Table 3. Incidence and Relative Risk of Ischemic Cardiovascular Disease and Myocardial Infarction in PI^{A1}/PI^{A2} Heterozygotes or PI^{A2}/PI^{A2} Homozygotes Versus PI^{A1}/PI^{A1} Noncarriers by Cox Regression, Stratified by Age

				Ischemic Cardiovascular Disease					Myocardial Infarction				
		Participants (n)	Hardy-Weinberg Equilibrium	First Events (n)	Incidence (95% CI)/ 10,000 Person-Years	Relative Risk (95% CI) Adjustment: None	Age	Multifactorial	First Events (n)	Incidence (95% CI)/ 10,000 Person-Years	Relative Risk (95% CI) Adjustment: None	Age	Multifactorial
Men													
<40 yrs	Noncarriers	999	p = 0.45	41	28 (21–39)	1.0	1.0	1.0	17	11 (6–18)	1.0	1.0	1.0
	Heterozygotes	414		20	33 (20–50)	1.2 (0.7–2.0)	1.2 (0.7–2.0)	1.1 (0.6–1.9)	6	9 (3–20)	0.8 (0.3–2.1)	0.8 (0.3–2.1)	0.8 (0.3–2.2)
	Homozygotes	37		5	102 (33–238)	3.7 (1.5–9.4)	3.6 (1.4–9.0)	3.0 (1.1–8.0)	3	56 (12–164)	5.4 (1.6–19)	5.2 (1.5–18)	3.8 (1.0–15)
40–50 yrs	Noncarriers	917	p = 0.72	137	93 (78–109)	1.0	1.0	1.0	66	41 (32–52)	1.0	1.0	1.0
	Heterozygotes	319		44	89 (67–122)	1.0 (0.7–1.4)	1.0 (0.7–1.4)	1.0 (0.7–1.4)	23	43 (27–65)	1.1 (0.7–1.7)	1.1 (0.7–1.7)	1.1 (0.7–1.8)
	Homozygotes	30		10	210 (101–386)	2.4 (1.3–4.6)	2.4 (1.3–4.6)	2.0 (1.0–3.9)	7	136 (55–280)	3.5 (1.6–7.5)	3.5 (1.6–7.5)	3.2 (1.4–7.1)
>50 yrs	Noncarriers	938	p = 0.65	292	176 (158–199)	1.0	1.0	1.0	132	72 (60–86)	1.0	1.0	1.0
	Heterozygotes	385		126	187 (156–222)	1.0 (0.9–1.3)	1.1 (0.9–1.3)	1.0 (0.8–1.3)	59	79 (60–101)	1.1 (0.8–1.5)	1.1 (0.8–1.5)	1.1 (0.8–1.5)
	Homozygotes	43		14	181 (99–304)	1.0 (0.6–1.8)	1.0 (0.6–1.8)	1.0 (0.6–1.8)	3	34 (69–98)	0.5 (0.1–1.4)	0.5 (0.1–1.5)	0.5 (0.2–1.5)
Women													
<40 yrs	Noncarriers	1,123	p = 0.61	19	12 (8–19)	1.0	1.0	1.0	6	3 (1–7)	1.0	1.0	1.0
	Heterozygotes	409		10	16 (8–30)	1.4 (0.6–3.0)	1.4 (0.7–3.0)	1.1 (0.5–2.5)	5	8 (2–18)	2.2 (0.7–7.1)	2.2 (0.7–7.2)	1.3 (0.1–4.5)
	Homozygotes	41		0	0 (0–70)		No cases		0	0 (0–66)		No cases	
40–50 yrs	Noncarriers	1,036	p = 0.54	72	42 (33–53)	1.0	1.0	1.0	24	13 (8–19)	1.0	1.0	1.0
	Heterozygotes	399		32	47 (32–66)	1.1 (0.7–1.6)	1.1 (0.7–1.6)	0.9 (0.6–1.4)	8	11 (5–22)	0.8 (0.4–1.8)	0.8 (0.4–1.8)	0.5 (0.2–1.4)
	Homozygotes	43		2	26 (3–95)	0.6 (0.2–2.6)	0.6 (0.2–2.6)	0.6 (0.1–2.3)	2	25 (3–90)	1.9 (0.5–8.1)	1.9 (0.5–8.1)	2.4 (0.5–11)
>50 yrs	Noncarriers	1,391	p = 0.61	256	96 (86–108)	1.0	1.0	1.0	87	30 (24–37)	1.0	1.0	1.0
	Heterozygotes	571		97	88 (73–109)	0.9 (0.7–1.1)	0.9 (0.7–1.1)	0.9 (0.7–1.2)	32	27 (18–38)	0.9 (0.6–1.3)	0.9 (0.6–1.3)	0.9 (0.6–1.3)
	Homozygotes	54		8	76 (33–150)	0.8 (0.4–1.5)	0.8 (0.4–1.6)	0.7 (0.3–1.4)	1	9 (0–50)	0.3 (0.04–2.1)	0.3 (0.04–2.1)	0.3 (0.04–2.1)
Age·genotype interaction test													
Men	Heterozygotes vs. noncarriers					p = 0.85	p = 0.87	p = 0.93			p = 0.84	p = 0.84	p = 0.79
	Homozygotes vs. noncarriers					p = 0.03	p = 0.04	p = 0.08			p = 0.002	p = 0.002	p = 0.004
Women	Heterozygotes vs. noncarriers					p = 0.47	p = 0.47	p = 0.89			p = 0.37	p = 0.37	p = 0.58
	Homozygotes vs. noncarriers					p = 0.64	p = 0.60	p = 0.64			p = 0.26	p = 0.26	p = 0.20

Multifactorial adjustment included all factors listed in Table 1.
CI = confidence interval.

Table 4. Age at First Event in PI^{A1}/PI^{A2} Heterozygotes, PI^{A2}/PI^{A2} Homozygotes, and PI^{A1}/PI^{A1} Noncarriers

	Ischemic Cardiovascular Disease		Myocardial Infarction	
	Age at First Event (yrs)	p Value	Age at First Event (yrs)	p Value
Men				
Noncarriers	67 ± 0.5	—	66 ± 0.5	—
Heterozygotes	66 ± 1.0	0.66	67 ± 1.0	0.83
Homozygotes	64 ± 2.0	0.07	61 ± 2.5	0.03
Women				
Noncarriers	71 ± 0.5	—	70 ± 1.0	—
Heterozygotes	71 ± 1.0	0.72	69 ± 1.5	0.80
Homozygotes	74 ± 2.5	0.47	66 ± 0.5	0.34

Age shown as mean ± SE. The p values are by Mann-Whitney *U* test versus noncarriers.

platelet GP IIb/IIIa PI^{A2}/PI^{A2} compared to noncarrier PI^{A1}/PI^{A1} young men over 22 years of follow-up in a large Danish cohort.

The more than 30 case-control studies published about this polymorphism and risk of ischemic cardiovascular disease reach divergent results (3,4). Two factors complicate a direct comparison of our results with the findings of others. Most importantly, the majority of previous studies combine PI^{A1}/PI^{A2} heterozygotes and PI^{A2}/PI^{A2} homozygotes versus PI^{A1}/PI^{A1} noncarriers in their calculations in order to obtain sufficient statistical power, and thus do not study homozygotes alone. Second, most previous studies did not investigate associations stratified on gender or age, which proved essential in our work. Our study suggests an association between homozygosity and ischemic cardiovascular disease in men, but not in women. This association is most pronounced in men <40 years of age, an age group where very few women are affected. Among the group 40 to 50 years of age, we do find cases among women and also an elevated relative risk for ischemic cardiovascular disease in homozygous men, but not in homozygous women, thus supporting the gender-specific association.

Contrary to most other reports, we do not find any increased risk of ischemic cardiovascular disease among PI^{A1}/PI^{A2} heterozygotes versus PI^{A1}/PI^{A1} noncarriers. Several factors could contribute to this discrepancy: publication bias toward positive results in small studies (4), different ethnicity between studies, differences in study design, and chance alone. Our study is the largest so far and, together with the two other published prospective studies (5,6), agree that PI^{A1}/PI^{A2} heterozygosity does not increase the risk of ischemic cardiovascular disease.

Of the many in vitro studies examining the impact of the PI^{A2}-allele on platelet function, two (19,20) investigated all three genotypes separately. In these studies, PI^{A2} was associated with increased platelet aggregability, and thus the propensity to initiate thrombus formation, in a gene-dose dependent manner. Biologically, the observation of increased risk of ischemic cardiovascular disease in PI^{A2}/PI^{A2} homozygotes therefore seems reasonable. However, this remains an untested correlation that may or may not explain the enhanced risk of homozygotes reported in our study.

Interestingly, when exposed to physiologic concentrations of estrogen, the aggregability of PI^{A1}/PI^{A2} heterozygous platelets was inhibited more than PI^{A1}/PI^{A1} noncarrier platelets (21). This accords with our data, which demonstrate an association between PI^{A2}/PI^{A2} homozygosity and ischemic cardiovascular disease in men only.

Prothrombotic conditions like increased platelet aggregability is believed by some investigators (22,23) to outweigh the importance of atherosclerosis in premature MI, mainly because of their findings of fewer stenoses on coronary angiography in young patients when compared with old patients with MI. Therefore, having the above-mentioned in vitro studies on PI^{A}-genotype and platelet aggregability in mind (19–21,24), our finding of age-dependency in men of the effect of the PI^{A2}/PI^{A2}-genotype on MI could simply be caused by increased platelet aggregability induced by the PI^{A2}/PI^{A2}-genotype operating at an early age.

In a series of autopsy studies on men, Mikkelsson et al. (25–28) reported lower incidence of atherosclerotic lesions but higher incidence of coronary thrombosis among PI^{A2}-carriers than among PI^{A1}/PI^{A1} noncarriers for participants <60 years, but not for those >60 years. Although the stratification on genotype and age in these studies (age under or above 60 years, genotype noncarriers vs. heterozygotes, and homozygotes combined) differs from our work, those studies nevertheless support our finding of higher risk of disease among younger homozygous men. Unfortunately, no data on the extent of atherosclerosis (including results from coronary angiographies) of the participants of our study are available. Therefore, we do not know whether the young homozygous men in our study have more or less atherosclerosis than expected.

We cannot totally exclude that our findings represent chance findings simply because no other studies have demonstrated this association in young men. However, the association was found in those <40 years old and in 40- to 50-year-old men, and the association decreased stepwise with increasing age.

Another potential limitation of our study is that participants underwent genotyping only if they attended the 1991 to 1994 third examination. The absence of a correlation between PI^{A2}/PI^{A2} homozygosity and ischemic heart disease in older men could be the result of a dropout of homozygous men at an early age because of premature death or disability. However, the stable Hardy-Weinberg equilibria throughout the age groups (Table 3) do not support such a hypothesis. Furthermore, age percentiles for noncarrier and homozygous men display a linear relationship, as would be expected with no selection against homozygotes (data not shown). Thus, we do not consider selection bias against homozygotes very likely, but if this does apply for our study, it would rather result in a conservative estimate concerning association of the PI^{A2}-genotype with ischemic cardiovascular disease, and thus cannot explain our results.

Systematic misclassification of genotypes in this study is unlikely, because of agreement with the Hardy-Weinberg equilibrium and because the PCR assay included a restriction enzyme site in each person assayed. Misclassification of ischemic cardiovascular disease in the Copenhagen City Heart Study is very low, as we have 99.97% follow-up, and because all hospital admissions as well as deaths are registered systematically throughout Denmark.

Medication given to prevent ischemic cardiovascular disease and MI might overcome an underlying genetic risk. Therefore, another limitation of the present study is that we do not have complete information on all preventive medication given to all participants during the entire 22-year follow-up; however, addition of the use of antihypertensive and cholesterol-lowering medications at the 1991 to 1994 examination to the multifactorially adjusted models only resulted in trivial changes in the RR estimates.

Finally, the main finding of our prospective study in the Copenhagen City Heart Study, with a total follow-up of 163,987 person-years, is the three-fold and four-fold 22-year risk of ischemic cardiovascular disease and MI in men <40 years homozygous for platelet GP IIb/IIIa Pl^{A2}/Pl^{A2}.

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